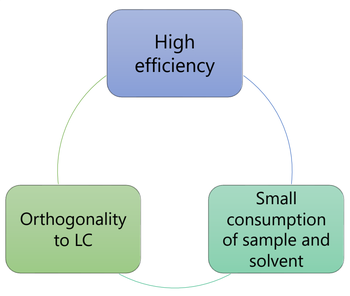
Proteomic analysis using capillary electrophoresis hyphenated with high resolution mass spectrometry: comparison of two coupling interfaces

Marie-Jia Gou, Gwenaël Nys, Gaël Cobraiville, Alice Demelenne, Anne-Catherine Servais, Marianne Fillet

Laboratory for the Analysis of Medicines, Department of Pharmacy, CIRM, University of Liège

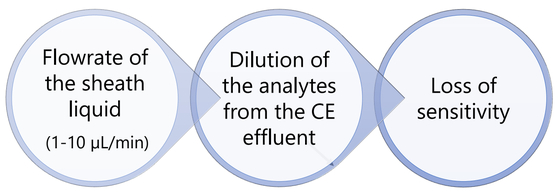
Introduction

Capillary electrophoresis tandem mass spectrometry (CE-MS/MS) is gaining interest in proteomics analysis and its use might bring complementary information in the analysis of complex proteome samples



CE and MS hyphenation is **not straightforward** due to the need of two independent electrical circuits to ensure in-capillary separation and spray formation.

→ Use of a **sheath liquid** to allow separation and stable spraying



Aims of the study

Sample enrichment and signal intensity

- Dynamic pH junction (DPJ) as online preconcentration technique
- Composition of the sheath liquid to enhance signal intensity

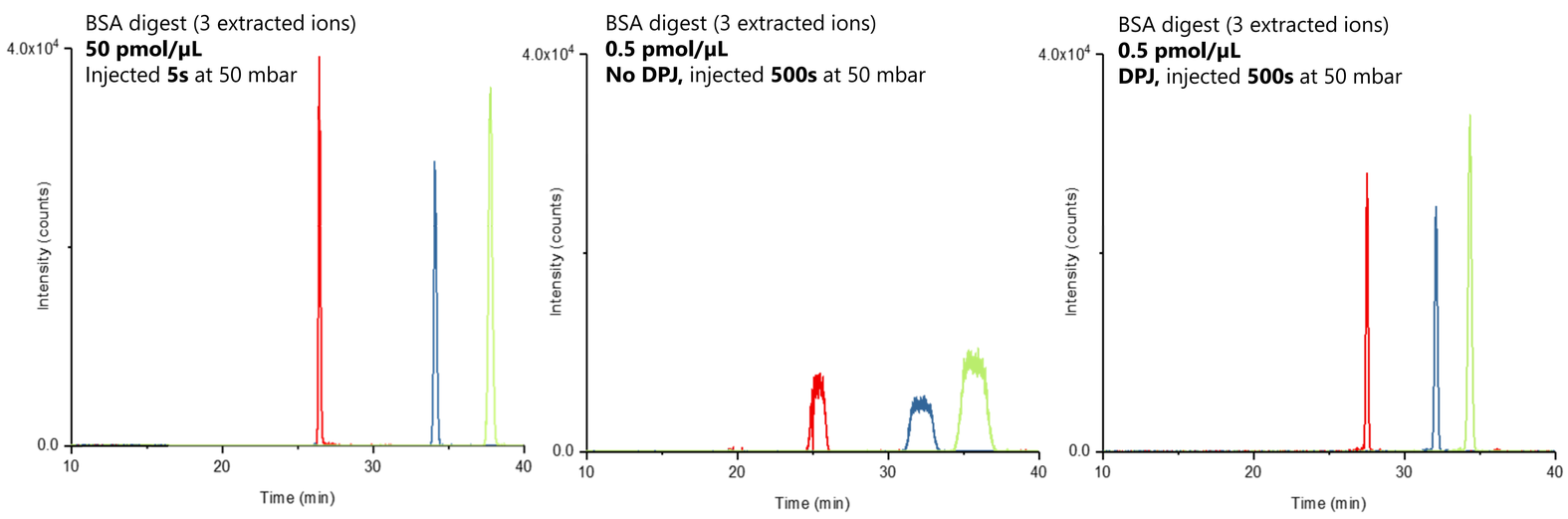
CE-MS coupling interfaces

- « Triple tube » as a microflow sheath liquid interface
- « EMASS-II » as a nanoflow sheath liquid interface
- Use of two complementary MS acquisition modes (DDA and DIA)

Comparison

- Number of identified peptides and proteins using a complex proteome digest
- Gain of sensitivity due to the use of a nanoflow interface

Results



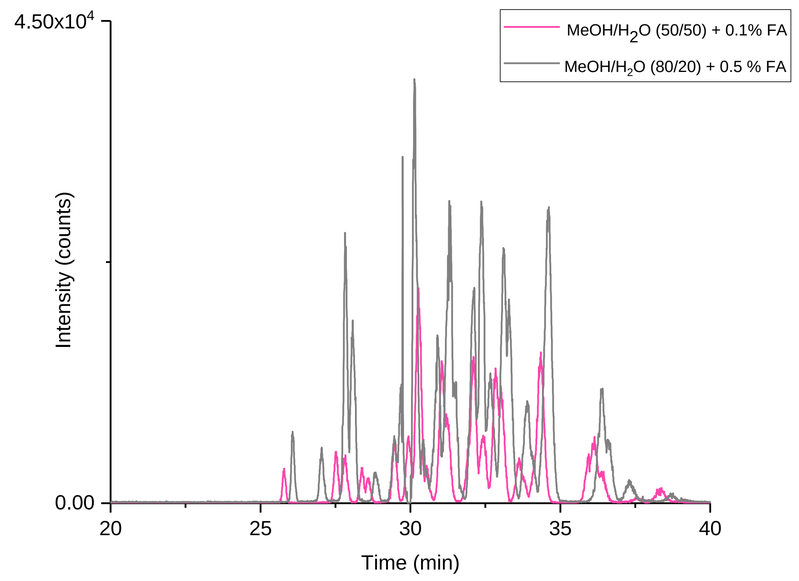
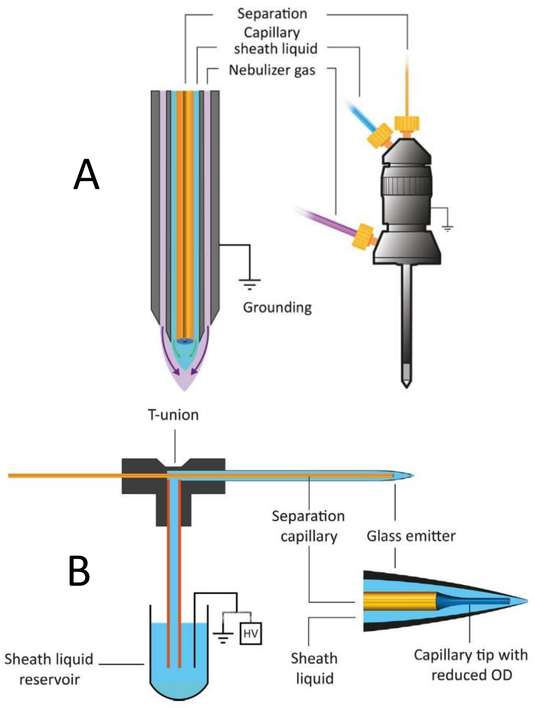


Fig.1: Effect of **dynamic pH junction (DPJ)** on peak intensity and peak width. The use of this online preconcentration technique to analyze a bovine serum albumin (BSA) digest allowed the improvement of **peak intensity** by approximately **100-fold**. Moreover, we were able to **inject a large amount of sample** (≈ 14% of the total volume of the capillary) and obtain satisfying peak width;

Fig.2: The **composition of the sheath liquid** greatly influences the **MS signal intensity**. It is usually composed of a mixture of **organic and aqueous** solution. Effect of the sheath liquid composition on signal. This figure shows the influence of the percentage of methanol (MeOH) as well as formic acid (FA) on signal intensity (BSA digest 0.5 pmol/µL)



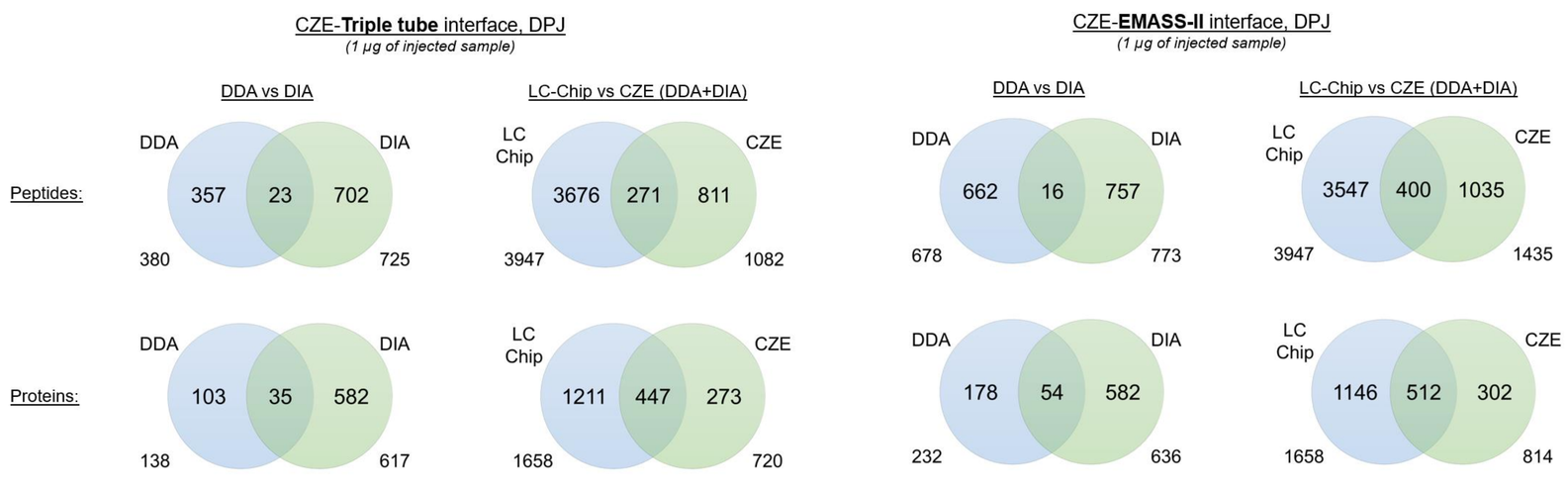


Fig.3: Diagram of the two CE-MS coupling interface namely the **Triple tube** as the **microflow** sheath liquid interface (A) and the **EMASS-II** as the **nanoflow** sheath liquid interface (B).

Fig.4: The number and uniqueness of the peptides and proteins identified by injecting **1 µg of *E.coli* digest** was evaluated. Two MS acquisition modes (**DDA and DIA**) were performed and the results obtained with **Triple Tube interface** (**microflow**, left) and **EMASS-II interface** (**nanoflow**, right) are shown in the Venn diagrams. The **complementarity of CZE with LC-Chip** is also demonstrated.

Conclusion

- The use of DPJ as preconcentration method allowed to improve 100-fold the sensitivity without sacrificing separation efficiency and peak width.
- The composition of sheath liquid is of great importance to enhance the signal intensity.
- The use of EMASS-II interface (nanoflow) allowed the identification of more peptides and proteins.
- The comparison of LC-Chip and CZE showed a great complementarity resulting in a larger coverage of the proteome.
- An improvement of 4-fold and 6-fold in peak area and peak height were observed with the use of a nanoflow sheath liquid interface.
- Despite the delicate handling and lack of automatization of the EMASS-II interface, it can be considered as an useful tool in proteomics research in terms of coverage and sensitivity improvement.

RÉGION WALLONNE · FONDS LÉON FREDERICQ · fnrs LA LIBERTÉ DE CHERCHER

CIRM ULiège

WEBSITE: www. cirm.uliege.be

CENTER FOR INTERDISCIPLINARY RESEARCH ON MEDICINES